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Non-Correlating Pap Tests: Histological Follow-up of Abnormal Pap Tests Classified Using the 2001 Bethesda System

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Abstract. The purpose of this report is to study the correlation between abnormal cervical Pap smears reported using the revised 2001 Bethesda system and their corresponding cervical biopsies, in order to identify discrepant non-correlating positive smears and analyze the smear characteristics causing the discrepancy in those cases. All abnormal cervical smears results classified using the revised 2001 Bethesda system and their follow-up cervical biopsies were retrieved and correlated. The total number, percentage and smear characteristics of all non-correlating positive Pap smears are presented. One hundred and thirty seven (137) (85 positive and 52 atypical but not positive) abnormal smears had follow-up cervical biopsies. The 85 positive smears were further studied and an exact cyto-histological correspondence was seen in 58 (68.23%) out of the 85 positive smears, while 27 (31.7%) smears were discordant. Nine (10.58%) out of the 27 discordant cases revealed a more severe lesion on follow-up biopsy, 7 (8.23%) cases with a less severe lesion and 11 (12.94%) smears were falsely positive. This study shows that concordance rate of cervical Pap smears reported using the revised 2001 Bethesda system with their follow-up biopsies is moderate and increases with the rise of the cytological grade of dysplasia.

Keywords: Pap smear, Abnormal, Cervical intraepithelial neoplasia, Cancer, Bethesda system 2001.

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Introduction

Cervical cancer is still considered a major health problem worldwide[1,2]. The introduction of Pap tests in the late 1940s has led to a dramatic decline in the incidence of this cancer in countries with well established cervical screening programs[2,3]. The last decade witnessed the development of new technologies in the processing of cervical material[4] and a genuine effort at standardization of Pap smear reporting through the introduction and later modification of the Bethesda system of Pap smear reporting[5,6]. This has had a great impact on the correct diagnosis and hence management of cervical intraepithelial abnormalities and invasive cancer.

Correlation of cervical cytology findings with follow-up biopsies is considered an important component of quality improvement programs in many cytopathology laboratories. Documented cytologic-histologic review has also been a requirement of the College of American Pathologists (CAP) laboratory accreditation program[7].

Although there are many internationally published reports,[8-13] not many are available from this part of the world describing this cytohistological correlation and the biopsy outcome of abnormal Pap smears reported using the revised Bethesda system. This study is conducted to assess the accuracy of Pap smears using the Bethesda system, in diagnosing cervical epithelial abnormalities in Saudi Arabia by correlating all abnormal Pap smears reported at King Abdul-Aziz Medical City (KAMC), Jeddah with their follow-up cervical biopsies, followed by identification of non-correlating discrepant cases and analysis of the smear characteristics in these discrepant cases.

Materials / Methods

This is a retrospective review of all Pap smears reported by the pathology department of KAMC/Jeddah in the period from January 1998 to August 2005.

A search in the computerized database of the pathology department for results of all conventional type Pap smears in the study period was carried out. The collected data was analyzed and the total number of sufficient Pap smears was first identified. The total number of abnormal smears defined according to the revised 2001 Bethesda reporting system as either harboring atypical squamous or glandular cells of undetermined

significances (ASC-US, ASC-H or AGUS respectively) or reported as positive for low or high-grade intraepithelial lesions (LSIL or HSIL respectively) or invasive cervical carcinoma were identified. Cases that were reported using older classification systems such as CIN terminology or the older 1990 version of the Bethesda system, the original cytology slides were retrieved and reviewed by an independent cytopathologist and reclassified using the revised 2001 Bethesda system.

All abnormal cervical cytology smears with subsequent follow-up cervical biopsy results in our institutional databases were identified and further analyzed. The cervical biopsy findings were classified as either inflammatory/reparative, changes related to Human Papilloma Virus (HPV), mild dysplasia (CIN I), moderate dysplasia (CIN II), severe dysplasia (CIN III) or invasive cancer. The abnormal cytology and its follow-up histology results were entered in an Excel spreadsheet.

The cytological diagnosis of abnormal but not positive (ASC-US, ASC-H & AGUS) has no histological diagnostic counterparts and hence was not included when applying statistical analysis for cytology/ histology correlation. All discrepant non-correlating cases, defined as smears that were positive on cytology for intraepithelial lesions or carcinoma but were either negative (false positive) or showed lower or higher grades of intraepithelial lesions (under diagnosed or over diagnosed respectively) on follow-up cervical biopsy were identified and further analyzed to identify the underlying smear characteristics leading to discrepancy. The underlying cytological errors were classified as either due to sampling error (air drying artifact or few cells), interpretation error and/or due to combined sampling and interpretation errors (such as excess blood or inflammation leading to false interpretation). The number and percentage of cases caused by each cytologic error was calculated and tabulated.

Results

The computer database records identified a total of five thousand seven hundred and forty six (5,746) cervical Pap smears reported in the Department of Pathology at King Abdulaziz Medical City in the period from January 1998 to August 2005. The study identified five thousand five hundred and ninety (5590) sufficient conventional type smears, and

one hundred and fifty six (156) insufficient ones. The latter cases were excluded from this study.

Out of the 5,590 sufficient smears, five thousand three hundred and twenty nine (5329, 95%) smears were reported as negative for intraepithelial lesions or malignancy and none of them had follow-up cervical biopsy. The abnormal smears constituted two hundred and sixty one (261, 5%) cases. Out of these abnormal smears, follow-up cervical biopsies were identified in one hundred and thirty seven (137) cases including 35 cases of ASC-US, 6 cases of ASC-H, 33 cases of LSIL, 22 cases of HSIL, 30 cases of invasive carcinoma, 10 cases of AGC, NOS, and one case of atypical endometrial cells, NOS. These constituted the basis for the present study. The follow-up histological material was as follows: ninety-nine (99) colposcopies directed biopsies, five (5) cervical cones and thirty three (33) hysterectomies.

The 137 available follow-up cervical biopsies in our data record were retrieved and reviewed. Pap smears with follow-up cervical biopsies were further studied and thirty cases were diagnosed by Pap smear as invasive carcinoma (21 squamous and 14 adenocarcinoma) had follow-up cervical biopsies and were proven on histology to be either true invasive carcinoma (29 cases, 96.7%) or only squamous carcinoma in situ with no histological evidence of invasion (1 case, 3.3%). The histological typing of the invasive carcinomas included thirteen (13) cases of squamous cell carcinomas, four (4) cases of endocervical adenocarcinoma, four (4) cases of metastatic carcinoma to cervix, and eight (8) cases of endometrial adenocarcinomas.

Twenty-two out of thirty-one cases reported as HSIL on Pap smear had follow-up cervical biopsy (see Table 1) and eight (36.3%) of the twenty-two were confirmed as high-grade dysplasia (CIN III) on histology while five (22.7%) were proven to be invasive carcinoma (i.e. under-diagnosed on Pap smear). The remaining nine cases were as follows: six cases were categorized as mild dysplasia (CIN I), one (1) case as moderate dysplasia (CIN II). The remaining two cases were identified as inflammatory/reparative changes (false positive Pap smears).

Thirty-three (33) of 56 cases diagnosed as LSIL on cytology had follow-up cervical biopsies (see Table 1). The histological diagnosis revealed six cases (18.2%) as HPV changes, 14 cases (42.2%) as CIN I, 3

cases (9.1%) as CIN II and one (3%) case as CIN III. The remaining nine (27, 3%) cases were inflammatory/reparative and negative for dysplasia or malignancy (false positive pap smears).

No invasive carcinoma was identified on follow-up histology of any LSIL Pap smears. The four cases diagnosed as LSIL on Pap smears and verified as high-grade lesion on biopsy (CIN III) were considered as under-diagnosed on Pap smears.

On the other hand, the single case of invasive cancer on Pap smear which was verified on follow-up cervical biopsy to be carcinoma in situ and the six cases of HSIL which proved on histology to be mild dysplasia (CIN I) were considered over-diagnosed on Pap smear.

Thirty-five (35) out of one hundred and three (103) cases diagnosed as ASC-US on Pap smears had subsequent follow-up cervical biopsy (see Table 1) and were proven histologic to be as follows: one case (2.85%) with HPV changes, four (11.4%) CIN I, one (2.85%) CIN II, three (8.57%) CIN III, and one (2.85%) of invasive carcinoma.

Table 1. Cyto-histologic correlations of all abnormal pap smears (n: 261).

	Histology								
Cytology	**Total**	**Total with biopsy**	**Inflamm.**	**HPV**	**CIN I**	**CIN II**	**CIN III**	**Carcinoma**	**No histo**
ASC-US	103	35	25	1	4	1	3	1	68
ASC-H	6	6	1				3	2	0
AGC, NOS	21	10	5	0	0	0	0	5	11
Atypical endomet., NOS	9	1	1	0	0	0	0	0	8
LSIL	56	33	9	6	14	3	1	0	23
HSIL	31	22	2	0	6	1	8	5	9
Invasive malignancy	35	30	0	0	0	0	1	29	5
Total	261	137	43	7	24	5	16	42	124

HPV: Changes consistent with Human Papilloma Virus.
CIN I, II, III: Cervical intraepithelial Neoplasia (Dysplasia) I, II, III.
ASC-US: Atypical squamous cells of undetermined significance.
ASC-H: Atypical squamous cells, cannot rule HSIL.
AGC, NOS: Atypical glandular cells and includes atypical glandular cells, none otherwise specified.
Atypical Endomet NOS: Atypical endometrial cells, none otherwise specified.
LSIL & HSIL: Low & High grade squamous intraepithelial lesions.

The remaining 25 ASC-US cases (71.42%) showed inflammatory/ reparative changes on follow-up cervical biopsies and were negative for dysplasia or malignancy.

All six cases diagnosed as ASC-H on Pap smear had follow-up cervical biopsies and were proven on histology to be as follows: three (50%) as severe dysplasia/carcinoma in situ, two (33.33%) as frank carcinoma and one case (16.7%) as negative for dysplasia or malignancy. The cytological material related to this last case was re-screened and the atypical changes in smears were interpreted as reparative changes secondary to radiation. Ten of 21 cases of atypical glandular cells, not otherwise specified (AGC, NOS) had follow-up biopsy and five (5, 50%) cases were proven histologically to be adenocarcinoma while the remaining 5 cases (5.50%) were chronic cervicitis. The single case diagnosed on Pap smear as atypical endometrial cells, NOS had follow-up hysterectomy and showed secretory endometrium with hormone therapy changes on histological examination. The cervix however was unremarkable. The association between Pap smear and follow-up cervical biopsy was statistically significant (Spearman's $p = 0.20$, $P < 0.04$)

Table 1 outlines the cyto-histological correlation of all abnormal Pap smears.

In order to further study the discordant non-correlating Pap smears, the fifty-two (52) cases reported as abnormal but not positive Pap smears (*i.e.* all cases diagnosed as ASC-US, ASC-H, AGC, NOS, atypical endometrial cells, NOS) were excluded from any further analysis.

Twenty-seven (27, 31.7%) out of the eighty-five (85) histologically verified positive Pap smears were labeled as discordant, non-correlating Pap smears and were as follows: eleven (12.94%) false positive Pap smears, nine (10.58%) cases of under-diagnosed Pap smears and seven (8.23%) cases of over-diagnosed smears. Table 2: number & percentages of discrepant non correlating histologic verified positive Pap smears ($n = 27$) with classification according to the underlying categories. One case was shown to be discrepant due to sampling error, fifteen cases due to interpretation error and eleven (11) cases due to combined sampling and interpretation errors.

Table 2. Number & percentages of discrepant non-correlating histologically verified positive pap smears (n=27) with classification according to the underlying categories.

Underlying etiologic factors for discrepancy				
Discrepant pap smear	**Cytologic sampling error N**	**Cytologic interpretation error N**	**Combined sampling & interpretation errors N**	**% of total no. of histologically verified positive smears N=85**
False positive	0	8	3	11(12.94%)
Under-diagnosed	1	5	3	9 (10.58%)
Over-diagnosed	0	2	5	7 (8.23%)
Total	1	15	11	27 (100%)

Discussion

Cervical cancer continues to be a major health burden especially in underdeveloped nations such as Latin America and Southeast Asia[1-3] where it represents the second most common cancer in females, after breast cancer[1]**.** The introduction of Pap test by Papanicolaou in the late 1940s has led to a dramatic decline in the incidence of this cancer in countries with well-established cervical screening programs such as United States, Canada and Europe[2]. In addition, the last decade has witnessed the development of new technologies such as HPV, DNA testing and liquid base cytology,[4] as well as standardization of Pap smear reporting through the introduction of the Bethesda system[5] which has had a great impact on correct diagnosis and management of cervical cancer. In 2001, a revised version of the Bethesda system was introduced to include new technologies and to simplify the smear reporting by eliminating vague clinically irrelevant terminology[6].

High rates of false negative and positive results affect the accuracy of Pap tests. One suggested method to increase its sensitivity and diagnostic utility is to correlate the findings on Pap smears with the one on follow-up cervical biopsies and to analyze the factors which hamper the correct cytological diagnosis. At present this type of cervical cytologic-histologic correlation is considered an important component of quality improvement programs in many cytopathology laboratories. Documented cytologic-histologic review is now a requirement of the CAP laboratory accreditation program[7].

In spite of several large international studies addressing this issue[8-13], not many reports are available from this part of the world describing cervical cyto-histological correlation and the biopsy outcome of Pap smears reported using the revised 2001 Bethesda system.

In the present study, fifty-eight (68.23%) out of the eighty-five positive smears showed exact correspondence while twenty-seven (31.7%) were discordant and revealed nine (10.58%) cases with a lesion more severe on follow-up biopsy, seven (8.23%) cases with a less severe lesion and eleven (12.94%) smears were falsely positive. When comparing the current study with similar internationally published larger studies, the findings differ slightly. Massad *et al.*[9] reviewed 1842 smears with squamous cytologic abnormalities and their follow-up cervical biopsies. The biopsy revealed a lesion more severe than that suggested by cytology in 577 (31%) cases, less severe lesion in 648 (35%) cases and exact correspondence in only 646 (35%) cases. Mathers *et al.*[10] presented the histological follow-up of 395 cervical smears over a period of six years. In that study, the positive predictive value of Pap smear increased with the degree of abnormality which is in agreement with the findings in the current study as demonstrated by the reduction of number of discrepant Pap smears with the increase in severity of cervical lesion. Our findings are also supported by the data presented by Takezawa *et al.*[11,12] who reviewed the correlation between abnormal Pap smears reported using the Bethesda system and their follow-up cervical biopsies. They found that squamous intraepithelial lesions were found in 10-79% of ASCUS. Smears reported as LSIL showed negative histology in 9-50% of cases and CIN II-III in 2-40% of cases. Benign findings were found in 6-43% of cases of women with high-grade SIL while 29-72% of HSIL were associated with CIN II-III and 0-2% with cancer. Similarly Jones and Novis[13] in a survey of 348 laboratories by the CAP, found squamous lesions in 41% of women with AGUS, 60% of those with ASCUS, 68% of those with LSIL, 95% of those with HSIL and 96% of those with cancer with substantial inter-laboratory variation in the degree of association.

In the present study, minimally abnormal cytological diagnoses such as atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesions revealed a wide range of squamous abnormality on follow-up cervical biopsy ranging from chronic cervicitis to high-grade dysplasia or even invasive carcinoma. This finding is

supported by similar international studies[14-16]. Zuna *et al.*[14] and Gupta and Sodhani[15] respectively reviewed non-correlating Pap smears and found that a substantial number of histological verified cases of severe dysplasia can have a smear interpretation of LSIL or less.

Lonky *et al.*[16] studied 566 cases of high-grade dysplasia and 8 cancers on biopsy and identified that high-grade lesions was proceeded by LSIL cytology in 224 (39%), ASCUS in 220 (38.9%) and 6 cases of cancer were preceded by HSIL diagnosis.

In his study, Lonky *et al.*[16] suggested that we should rethink the significance of minimally abnormal Pap smears when designing triage protocols that delay or eliminate colposcopy based on the Bethesda system alone. It seems logical that based on the present, as well as previously published data[17-19], that colposcopic examination with direct punch biopsy of any abnormal colposcopy lesion is recommended for all women with a cytologic diagnosis of ASCUS and LGSIL. Elimination or reducing the use of the ASCUS appears to decrease the sensitivity of the Pap test significantly and appears to reduce the chance of predicting the diagnosis of SIL on biopsy including HSIL[17]. On the other hand, the addition of the term ASC-H (atypical squamous cells, high-grade lesion cannot be excluded) to the ASC diagnostic category in the revised Bethesda system helped segregate those cases with a higher probability of having high-grade dysplasia on follow-up biopsy[6].

Analysis of discrepant smears in order to identify factors hampering the recognition of the true severity was carried out in this study. The underlying etiologies included lack of consistency among pathologists for the interpretation of metaplastic patterns and specimen adequacy errors, particularly air drying artifact. Other previously published studies supported these findings[15,16,20].

Tritz *et al.*[20] investigated all possible etiologies for non-correlating cervical cytologies and follow-up biopsies by assigning an etiologic category for each of these cases. These included biopsy or cytologic sampling, cytologic screening, histotechnical processing or cytologic or histological interpretation errors and found that the most common causes for discrepancy was colposcopic biopsy sampling (36 cases, 51%) and cytologic sampling (11%). The use of an appropriately designed method of reporting the causes of discrepancy similar to the one in the current study is suggested since it allows identifying problem areas and design

specific corrective actions, which will improve diagnostic accuracy and eventually patient care.

Excess blood or inflammation may mask high-grade cells and lead to under-interpretation. In addition, this study as well as other findings[21] showed that poor quality specimen and inadequate sampling might contribute to false negative results or under-diagnosis. Proper Pap smear technique with immediate fixation to avoid air drying artifact as well as careful slide screening and abiding to strict adequacy criteria will help decrease this problem.

In the Q-probe study carried out by the CAP[13], the major discrepancies were attributed to cytology or biopsy sampling errors. The study concluded that routinely providing the patient's recent cervical cytology report to the surgical pathologist at the time of biopsy examination would result in improved sensitivity.

The prevalence of endocervical adenocarcinoma and its precursors has increased, partly due to increased awareness of these lesions. Having said that, the atypical glandular cells of undetermined significance (AGUS) category presented in the previous 1991 version of Bethesda often causes diagnostic uncertainty in cervicovaginal smears since patients with a diagnosis of AGUS in Pap smears can represent a variable spectrum of underlying pathologies ranging from chronic cervicitis, LSIL, adenocarcinoma in situ (AIS) and even invasive carcinoma[10,22-24]. In the revised 2001 Bethesda system[6], the AGUS diagnostic category was eliminated and to prevent confusion with ASCUS, pathologists were further requested to comment not only on the level of pleomorphism (AGC, NOS versus AGC, favor neoplastic) but also to make an effort to identify the origin of the glandular cells (endocervical versus endometrial). The positive predictive value of AGC for high-grade lesions was shown to be higher than ASC in one study[23] wherein 48% had either SIL or adenocarcinoma.

In a more recent study[24], the authors showed that 19% of smears reported as AGC, NOS had significant pathology and suggested that although the proportion of patients having significant underlying pathologies than AGC, favor neoplastic where 68% had significant pathologies, smears showing AGC, NOS still warrant early investigation and follow-up. In spite of a lower number of patients studied, similar results were obtained in the present report where 50% of AGC, NOS

proved histologically to be adenocarcinoma while the other 50% were chronic cervicitis. The single case diagnosed on Pap smear as atypical endometrial cells, NOS had follow-up hysterectomy and showed secretory endometrium with hormone therapy changes on histological examination. The cervix, however, was unremarkable. Larger numbers of AGC smears should be studied especially from this part of the world in order to be able to give solid recommendations.

The fact that more than one-third (96 cases) did not have a record of follow-up, cervical biopsy may hinder the study of concordance incomplete. This lack of follow-up biopsy, especially in positive cases is mainly because of patients' poor compliance, but it is also because of a lack of available, standardized protocol followed among different physicians regarding whether or not to request repeat cytology, perform colposcopy and biopsy or even look for other alternatives such as HPV-DNA testing, (not available in our center), especially in cases of ASC-US. Education of patients and close follow-up with clinicians by using a reminder computer system is recommended in order to make the most of such a very successful screening program.

Conclusion

In conclusion, this study from the western region of Saudi Arabia and in agreement with published international figures confirms that the concordance rate of cervical cytology results reported, using the revised Bethesda system, with their follow-up biopsy is moderate and increases with the rise of the cytological grade of dysplasia. Major underlying etiologies for discrepancy include specimen adequacy, air drying artifact and lack of consistency between pathologists in diagnosis of minor Pap smear abnormalities.

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مسحات عنق الرحم غير المتطابقة: متابعة مسحات عنق الرحم الشاذة المصنفة حسب نظام بيثيسدا ٢٠٠١ عن طريق فحص العينة المجهري

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جـــدة – المملكة العربية السعودية

المستخلص. تَمَّ دراسة الارتباط بين مسحة عنق الرحم الشاذة المصنفة حسب نظام بيثيسدا وفحوص العينات المستخرجة من عنق الرحم لنفس الحالات، لكي نميز المسحات الإيجابية المخالفة وغير المتطابقة، ونحلل خصائص المسحة في تلك الحالات، صنفت كل نتائج مسحة عنق الرحم الشاذة وفحوص العينة العنقية التابعة لها ثم فصلت المسحات الموجبة وغير المتطابقة، وتم دراسة خصائص المسحة والنسبة المئوية لكل مسحة غير متطابقة، وتبين أن مائة وسبعة وثلاثين (١٣٧) مسحة عنق رحم (٨٥ إيجابيًا و ٥٢ شاذ لكن ليس إيجابيًا) توبعت بفحص عينة لعنق رحم. المسحات الإيجابية كان عددها خمسة و ثمانون (٨٥) وأن ثمانية وخمسين (٦٨,٢٣٪) مسحة من مجموع الخمسة وثمانين مسحة إجابية كانت متطابقة، بينما سبعة وعشرون (٣١,٧٪) مسحة كانت مخالفة. تسعة حالات (١٠,٥٨٪) خارج الحالات المخالفة السبع والعشرين كشفت نتيجةً أكثر خطورة، وسبعة (٨,٢٣٪) أقل خطورة من نتيجة مسحة عنق الرحم، في حين إحدى عشر (١٢,٩٤٪) مسحة كانت إيجابية بشكلٍ خاطئ . تبين هذه الدراسة أن نسبة التطابق بين نتائج مسحة عنق الرحم والعينة العنقية التابعة لها متوسطًا ويزيد بزيادة درجة النمو الشاذ في عنق الرحم.